XIV.—*Contributions to the Knowledge of the* Amœbæ. By Dr. August Gruber*.

[Plate IX.]

Auerbach†, as is well known, starting from the assumption that a membranous boundary was a necessary attribute of a cell, set up a theory, quite intelligible under the circumstances of the time, according to which the *Amœbæ* also, as unicellular creatures, had a membranous envelope. This opinion was refuted by subsequent naturalists; and it was Greeff‡ principally who gave a more correct interpretation of Auerbach's observations. With the overthrow of this theory, however, some forms of *Amœbæ* and many of the phenomena of their sarcode-body, well known to and very distinctly figured by Auerbach, although not quite rightly interpreted by him, seem to have been thrown into the background.

This refers to two *Amœbæ* whose bodies appeared to be surrounded by a double-contoured fine envelope, and which were described under the names of *Amœba bilimbosa* and *Amœba actinophora*. They were afterwards again mentioned by Hertwig and Lesser§, and regarded as identical with their *Cochliopodium*, which, however, as I shall hereafter show, can hardly be the case. We must also accept similar conditions for Greeff's || genus *Amphizonella*, as distinctly appears from his figure (fig. 18) of the colourless species.

The existence of a fine layer of clear protoplasm round the *Amœba*-body, which must be penetrated by the pseudopodia, seems to me to be by no means an insignificant phenomenon; and I hope to excite some interest by citing another form of *Amœba* of the same kind and by a fresh investigation of Auerbach's *Amœba actinophora*.

1. *Amœba tentaculata*, sp. n.

I found the *Amœba* which forms the subject of the following consideration in the small sea-water aquarium of the Zoological Institute here [Freiburg im Br.]. The water and the vegetable and animal organisms contained in it are chiefly derived

* Translated from the 'Zeitschrift für wissenschaftliche Zoologie,' Band xxxvi. pp. 459–470.

† Auerbach, "Ueber Einzelligkeit der Amöben," Zeitschr. f. wiss. Zool. Bd. vii.

‡ Greeff, 'Ueber einige in der Erde lebenden Amöben und andere Rhizopoden.'

§ Hertwig und Lesser, "Ueber Rhizopoden und ihren verwandte Organismen," Arch. f. mikr. Anat. Bd. x. Supp.

|| Arch. f. mikr. Anat. Bd. ii.

from the Frankfurt aquarium. But this spring I brought with me some bottles of sea-water with living contents from the Baltic coast and from the harbour of Genoa, and mixed this with the rest, so that I am by no means in a position to furnish the habitat of the creature that will here be described. The marine Protozoa, or at any rate those of the coast-fauna, seem, however, to be tolerably cosmopolitan; and we may therefore assume for *Amœba tentaculata* a wide distribution in our seas*. If I beat fragments of a seaweed upon the object-slide, or scraped off a little of the crust deposited on the glass wall of the aquarium, some specimens of the *Amœba* almost always made their appearance.

It forms a little mass of very variable size. The smallest examples measured about 0·03 millim., the largest 0·12 millim.

In consequence of its greater refractive power, the body stands out luminously from the water, a property which, in the protoplasm of all Rhizopoda, goes hand in hand with greater viscosity. Here also we find the rule confirmed; for the protoplasm of *Amœba tentaculata* is, in fact, an extremely tenacious mass, in comparison with that of allied creatures.

Under a low power (about 80 diameters) we can see no movement or change of form in the animal; and it is only when we employ high and very high powers that we can convince ourselves that we have before us an *Amœba* the form of which is engaged in a continual although sluggish change. We shall soon see that the apparently motionless animal is really capable of locomotion, and may pass into a flowing state, distinctly recognizable under high powers.

But if we first of all examine the creature in the resting state, in which it generally is when it has not long been placed on the object-slide, the *Amœba* has then essentially the same form as an *Amœba verrucosa*; i. e. the whole body is, as it were, shrunk together, with its surface covered with elevated knobs and deep folds, which slowly change their form and position.

In the interior the vital activity of the protoplasm is manifested by a streaming and trembling movement of the fine dark granules with which the sarcode is abundantly furnished.

So far there would be nothing remarkable to observe in the

* Last spring I found my *Cothurnia operculata* (Zeitschr. f. wiss. Zool. Bd. xxviii.) in the harbour of Genoa, whilst the former examples were derived from the Frankfort aquarium, and therefore from northern seas. The same aquarium also contained *Cothurnia socialis*, referred to as cited above; and this I quite recently discovered in abundance upon fragments of Hydrozoa brought from the Baltic.

behaviour of *Amœba tentaculata*, and its conditions would perfectly agree with those occurring in *A. verrucosa*, which is so abundant and has been so often described.

But while in the latter we miss true pseudopodium-formation, both in the resting state and during flow, we are surprised here by seeing fine protoplasmic filaments make their appearance at different parts of the body. These are thin processes of equal breadth throughout, which stand out from the body, sometimes in one place, sometimes in another, and bend to and fro as if feeling about, often curve into a bow, but generally remain extended pretty straight. It first struck me that these pseudopodia did not, as in other *Amœbæ*, spring from the protoplasmic body in the shape of fingers gradually becoming thinner, but that small conical elevations of the body served as their base, and that they rose from these with a distinctly marked separation. When such pseudopodia with their supports were very numerous, they gave the *Amœba* a very peculiar appearance, which I have attempted to represent in fig. 1.

The business now was to discover a reason for the peculiar behaviour of the pseudopodia; and in this I very soon succeeded by the employment of immersion systems (Hartnack, No. X. or Seibert homogeneous imm.). It proved that the whole *Amœba* is enveloped by a fine layer of denser substance, consequently a membranaceous cortical layer, which causes the periphery of all its humps and processes to appear distinctly double-contoured.

In the case of the terrestrial Rhizopods like *A. tentaculata* described by Greeff *, the idea of a similar tougher cortical layer could not be avoided, as also in that of *Amœba verrucosa*, which is so often mentioned. In the case of the latter, indeed, I did not succeed in detecting any thing of the kind; but Leidy † says, "A striking peculiarity of *Amœba verrucosa* is, that the outlines of the body, the pseudopodal expansions, and the wrinkles of the surface often appear defined with partial or interrupted double lines, as if the animal were invested with a delicate membrane (pl. iii. figs. 1, 2, 7, 28, 29)." It is, however, certainly such a membrane, or rather membranaceous thickening of a fine cortical layer, that we find in *Amœba tentaculata*.

Directly within this firmer envelope lies the soft internal sarcode-mass! If a pseudopodium is to be pushed forth, the enveloping layer must first be broken through. This, however, offers some resistence, and is consequently pushed out in

* Archiv f. mikr. Anat. Bd. ii.

† Leidy, 'Freshwater Rhizopods of North America,' p. 55.

a conical form*. An aperture is broken through at the apex of the cone; and the sarcode issues in the form of a thin filament. Fig. 8 may serve to illustrate this process; in it we see distinctly the thin cortical layer (*b*) of the pseudopodial cone, and also within it the central substance (*m*), which is pushed forth as a pseudopodium (*p*) at the apex.

I succeeded in observing very distinctly the retraction of the pseudopodium, after which a new one frequently issued from the same cone. I also believe that I have often seen the issue of two pseudopodia simultaneously.

The pseudopodial cones have a very constant form; and although they can obliterate themselves again completely, this does not always take place after the retraction of the pseudopodium; but very frequently the elevation persists afterwards, and a small crater seems to have been formed at the spot where the orifice for the pseudopodium was situated (see fig. 2, *k*). I once found a specimen on which there were many pseudopodial cones, but all without processes (fig. 4, *k*); nevertheless they persisted, without alteration, for a considerable time.

I said above that the pseudopodia which are produced in this way bend slowly to and fro, a movement which they have in common with those of other *Amœbæ*. Whether they act as tactile organs, or are destined to bring in food, I cannot definitely state. The former, however, appears to me more probable; for we find in the interior nutritive materials, such as Diatoms, Algæ, &c., which are much too large to be capable of penetrating through the narrow aperture of the pseudopodial cone †.

At any rate the animal, notwithstanding its firmer enveloping layer, is able to take in solid materials. Moreover we know very nearly allied forms, such as *A. verrucosa*, which are destitute of these organs, and nevertheless take in such nutritive bodies. Sometimes it appeared to me as if a slow locomotion was effected by means of the pseudopodia, but only to very inconsiderable distances.

In advancing *A. tentaculata* employs no special organ any more than its allies which possess a firm cortical layer. The form in which we have hitherto considered it characterizes only the resting state of the *Amœba*. We soon see movement

* Conical elevations have also been described in *Podostoma filigerum*. It is even said that a sort of buccal aperture occurs in them; but this requires confirmation. At any rate, they may be referable to the structures before us. Moreover protrusions of similar appearance may very probably occur in other *Amœbæ*. (See also Auerbach, *loc. cit.* fig. 15.)

† Somewhat as in *Podostoma*.

taking place in the main mass itself; the humps and folds gradually disappear, the pseudopodia are for the most part drawn in, and with them the cones; and after the surface has become smooth, there commences a steady flow in one direction, exactly in the same manner as has long been known in *A. verrucosa*, although much slower. In the latter this stage was for a time regarded as forming a distinct species under the name of *A. quadrilineata*.

The longitudinal folds which gave origin to this name, and which are produced by the strain on the tenacious outer layer acting in one direction, occur here just in the same manner (figs. 5, 6, & 7). Along them we see the granules hastening forward in several streams, whilst a clear mass of protoplasm, free from granules, in constant flow moves on before them. A remarkable circumstance is that on the leading part of the body pseudopodia with their cones frequently persist, and thus, to a certain extent, may act as extended feelers (fig. 7).

While at the posterior end, *i. e.* at the part opposite to that which is pushing forward, the double contour is distinctly preserved in the outer layer, it disappears entirely on the anterior part (fig. 6), from which we must conclude that the first-mentioned part of the body retains its toughness, whilst anteriorly all becomes in flux, *i. e.* the more fluid constituents collect there. Nevertheless even these still have considerable density, as is proved by the pseudopodia and pseudopodial cones protruded from them, on which, however, no double contour is visible. Frequently a zone of clear protoplasm seems to surround the whole body; and then the double lines are no longer seen anywhere.

Of a nucleus nothing is to be seen while the *Amœba* remains in the resting state and the folds of the surface obstruct the view of the interior. But if the Rhizopod begins to move, when the body flattens itself completely, the nucleus at once becomes distinctly visible (*n* in the figures), and appears as a little disk surrounded by a narrow border, as in most *Amœbæ*. No contractile vacuole is present, a new proof of the still unexplained fact that this structure is wanting in the marine Rhizopoda.

2. *Amœba actinophora*, Auerbach.

The Rhizopod that is to be described here is a very small *Amœba*, measuring 0·03–0·04 millim., which occurred pretty plentifully in all sorts of receptacles of water in the neighbourhood of Lindau. It excited my interest because it seemed to have much in common with the *Amœba tentaculata* that I had previously observed; and, in fact, it proved that it was exceedingly suitable for the completion and elucidation of the observations made on the latter.

I had already completed my observations and made the drawings which are here given before I could procure the literature which showed that the form in question was nothing else than Auerbach's *Amœba actinophora.*

A comparison of the figures given by this naturalist with mine shows how closely we are in agreement as to external characters; and that I have, notwithstanding, reproduced my drawings is in order that they may illustrate the point in which I differ from Auerbach, namely the behaviour of the outer membranous cortical layer, which here especially interests us. In accordance with this I also give the description in such a manner that it may represent the observation as I then made it, uninfluenced by any thing previously known.

The first striking point was that here also the protoplasm was distinctly surrounded by a double contour, and the animal appeared as if covered by an envelope.

The periphery was for the most part perfectly smooth, and only at one point did the animal extend a larger or smaller number of lobate pseudopodia. In this way the *Amœba* acquired delusively the appearance of a thalamophorous Rhizopod, with a closely-fitting thin carapace, from the orifice of which processes protruded. A glance at fig. 9 will explain this better than a detailed description. In this condition the protoplasm in the interior forms a tolerably compact mass, in which there are a number of rather large strongly-refractive granules.

When the number of the pseudopodia is large, so that a whole tuft of them protrudes at once (fig. 9), we see nothing of the cortical zone at their place of issue; it is entirely displaced. It is otherwise when only a few, say two or three, processes are pushed forth. The relations of the marginal layer are then quite distinctly visible, and we find that, just as in *A. tentaculata*, the cortex is pushed out into a cone, at the apex of which the pseudopodium makes its way out. Here, therefore, the double contour is also produced by a more tenacious layer surrounding the animal, which must be penetrated by the protoplasmic processes before they can issue (fig. 14). Even in the previously described form, however, we saw that we have not to do with a persistent membranous structure, but that during the flow of the animal the cortical layer becomes amalgamated with the rest of the sarcode. This is much more distinctly observable in *Amœba actinophora*. Thus all at once we see how, as the animal changes its form, the pseudopodia are at the same time nearly all retracted, the body becomes flattened, the cortical zone vanishes and flows into a broad border of clear protoplasm, which surrounds the darker richly granular mass in the centre of the animal (figs. 11 & 12, *h*). The latter often remains for some time sharply discriminated from the hyaline border (fig. 17); but the boundary is soon obliterated, exactly as during the formation of an ordinary pseudopodium (fig. 12). In this state the nucleus (*n*) also becomes quite distinctly visible, agreeing precisely in its structure with those of other *Amœbæ*.

The melting of the fine cortical layer into the broad clear border does not take place with equal rapidity at all points; so that a part of the *Amœba* often appears sharply limited, whilst another is already surrounded by the clear space (fig. 11, *r s*). In fig. 14, for example, is represented an *Amœba diffluens*, one side of which is already quite liquefied, while on the other half the double-contoured enveloping layer is still retained, and on it even two pseudopodial cones, with the processes issuing from them, are still visible. Fig. 15 is also instructive in another way. There the cortical layer has become fluid, and we see that the two pseudopodia which have persisted consist of the same hyaline protoplasm as the clear border in which the cortical zone previously sharply separated from it (see fig. 14) has dissolved itself. In the first state, therefore, there would have been an envelope and an endoplasm enclosed by it, and from which the pseudopodia proceeded, clearly distinguishable; in the latter both have become fused into one. Rapidly as the broad, scarcely visible border had formed, it can just as rapidly contract itself again; it shrinks to a certain extent together, until the narrow cortical layer again originates from it.

In this way *Amœba diffluens* can continually change its aspect completely in one or other of the modes described. Upon what law this power depends cannot be stated definitely; very probably, however, different conditions of pressure come into play in the matter. With a centripetal pressure acting uniformly upon the whole periphery, the more fluid parts of the protoplasm are all pressed into the interior, and only the narrow membranaceous boundary remains. This acquires a firmer consistence by contact with the water; and therefore at the points where pseudopodia issue it is pushed aside by the latter. If the general pressure ceases, the more fluid constituents again come forth from the interior, dissolve the solidified cortical layer, and form the clear border.

The best illustration of this explanation of the process is furnished by those cases in which a slow flowing forward of the *Amœba* in one direction is taking place (fig. 14). On the advancing side the fluid constituents are pushed on in front; here all pressure has ceased, whilst it acts upon the opposite side, where, accordingly, the cortical contours are quite distinctly to be seen.

Auerbach had also observed this liquefaction into a disk, as is shown by his fig. 8; but he conceived of it as a phenomenon of expansion in which the cell-membrane also had to take part; but we now know that no such membrane exists, and that the envelope is to be regarded only as a transitory concentration of the outermost layer of sarcode, and can at any time dissolve again (see fig. 11).

Taking into consideration some other forms belonging here, *Amœba bilimbosa* of Auerbach is the first to be mentioned. I do not think that it is identical with those just described; the very distinct figures given by the discoverer (plate xix.), the difference of size, and several other differences are opposed to such a notion. In this case nothing is said of a disappearance of the cortex; and this reminds us more of the conditions stated by Greeff (*loc. cit.*) to occur in his *Amphizonella digitata* (fig. 18).

Special interest also attaches to *Cochliopodium pellucidum* of Hertwig and Lesser *, which so closely resembles *A. actinophora* that, as already stated, its discoverers regarded it as identical with the latter. But if the description of Hertwig and Lesser is correct (and this can hardly be doubted in the case of such accurate observers), there can be no further question of a union of the two species. Thus the envelope of *Cochliopodium* represents a true carapace, which "shows a hatching perpendicular to the surface," and thus acquires a great resemblance to the carapace of an *Arcella*. From its firmness it cannot be perforated by pseudopodia, and it has only a wide aperture "opposite the cell-nucleus" for the issue of protoplasmic processes, which gives it perfectly the appearance of a monothalamian when it is looked at from the side (Taf. ii. fig. vii. A). In this position *Cochliopodium* would then correspond to my figure 9. But, singularly enough, a state also occurs, and is very distinctly figured by Hertwig and Lesser in their fig. vii. C, which exactly represents an *Amœba actinophora* when the cortical layer has liquefied on all sides (fig. 12).

Hertwig and Lesser explain the matter by supposing that the perfect disappearance of the envelope is only delusive, owing to the animal here being seen not from the side, but

* *Loc. cit.* pl. ii. figs. 7, 8.

from above and behind, whilst the clear border is due to the sarcode which has flowed out of the aperture situated beneath.

In *Amœba actinophora* this is certainly not the case, as I think I have shown distinctly enough, and as will be understood without further discussion by examining my fig. 11, in which the cortex only shows a few remains (*r s*), which have already completely disappeared in fig. 12; or my fig. 16, which represents the same example as fig. 9, which, without change of place, underwent the alteration under my eyes. The resemblance of *A. actinophora* to *Cochliopodium* is still further heightened when we see that the cortex also appears finely punctate or lined, which struck me especially on the addition of osmic acid (fig. 17). The hyaline protoplasm also then appears finely punctate; and the impression is produced as if the finest granules effected the liquefaction of the cortex by the reception of more fluid constituents between them.

A great similarity to the *Amœba* here described is presented by the Rhizopod represented by Hertwig and Lesser as a doubtful form in fig. 8 A, as will be seen from a comparison with my fig. 10. In this, however, the envelope (which is even of a yellowish colour) is evidently much thicker.

We may therefore demonstrate a perfectionation of this structure from *Amœba tentaculata*, through *A. actinophora*, to *Cochliopodium*. It might be conceived that by a further increased tenacity of the cortical zone we shall finally be led to those forms of monothalamous Rhizopods whose envelope forms only a soft membrane closely embracing the sarcode, and which is still so completely at one with the protoplasmic body as to accompany it in all its movements, and to be constricted simultaneously in the division.

Glancing back once more upon the phenomena which confront us in the Amœbiform Rhizopods surrounded by a distinct cortical zone, we shall find in them a welcome elucidation of conditions such as have only been guessed at in the case of other *Amœbæ*.

In the sarcode-body more fluid and less fluid constituents are present; the former we find at the spots which betray a centrifugal movement, whether in the pseudopodia or in the advancing part of the flowing *Amœbæ* (*A. quadrilineata*, *villosa*, *tentaculata*, &c.). The heavier constituents remain behind and are dragged along; and we see them finally break into many cushion-like processes of hyaline protoplasm.

The pushing forward of the more fluid constituents is effected by the action of a pressure upon the opposite side; this is produced by the outermost layer of protoplasm at this part acquiring a tougher consistency by extraction of water.

The latter is widened, during the flow of the *Amœba*, at the posterior end, by all sorts of processes, lobes, hairs, &c., which often give the *Amœba* a peculiar aspect, and have led to the establishment of distinct species*. The sarcode here becomes so tough that as the *Amœba* hastens forward it draws into threads, if the expression may be allowed.

If the direction of movement is reversed, the previous posterior extremity begins to flow, and the most tenacious protoplasm occurs on the opposite side. These conditions may be equally well studied on the lobate pseudopodia, as also during the retraction of the pseudopodium, on the surface of which all sorts of humps and folds are produced.

A tougher cortical zone of this kind is actually to be seen in the forms here under consideration. When there is a centripetal pressure acting uniformly, it surrounds the whole *Amœba* like a membrane; if the pressure ceases on all sides the *Amœba* flattens into a disk, the cortical zone liquefies and flows together into a clear border of more fluid sarcode; but if the pressure acts on one side, the liquefaction takes place only on the opposite side, and the mode of movement which may be called the flow of the *Amœba* is produced.

In the formation of individual pseudopodia (see *A. tentaculata*) it is only a few spots that are subjected to these conditions, and in accordance with this the tougher cortex dissolves only at certain points, making way for the issuing softer sarcode.

EXPLANATION OF PLATE IX.

Figs. 1–8 relate to *Amœba tentaculata*.

Fig. 1. An *A. tentaculata* with many pseudopodia.

Fig. 2. Another, 0·12 millim. long, under a higher power (Hartnack eyepiece 3, objective 10 immersion) and drawn with the *camera lucida*. It shows the cortical zone (*r s*), the pseudopodia (*ps*) on their cones, and at *k* a cone of which the pseudopodium has been retracted (crater).

Fig. 3. A portion of an *Amœba* with three pseudopodia, highly magnified.

Fig. 4. A specimen on which a number of craters (*k*) are to be seen.

Fig. 5. A specimen in which the cortical zone is dissolved.

Fig. 6. A flowing *Amœba tentaculata*, in which the nucleus (*n*) is very distinctly visible.

Fig. 7. Another, in which three pseudopodia (*ps*) are still retained on the advancing part.

Fig. 8 A. A pseudopodium with its cone. *m*, the soft interior mass; *b*, the cortex; *p*, the pseudopodium.

Fig. 8 B. A pseudopodium in course of being retracted.

Figs. 9–17 relate to *Amœba actinophora*.

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* These structures have recently been referred to by Engelmann (Onderz. Physiol. Lab. Utrecht, Deel vi. Afl. 2, St. 4).

Fig. 9. An *A. actinophora* with a distinct cortical layer (*rs*), and a tuft of pseudopodia at one end (Hartnack, oc. 3, obj. 7).
Fig. 10. Another with few pseudopodia, distinctly showing how they break through the cortex. (Rather too large in proportion to the following figures.)
Fig. 11. The same example a short time afterwards. The cortex (*r s*) is almost everywhere liquefied, and has become converted into a clear space (*h*): *n*, the nucleus, which is distinctly visible in this state.
Fig. 12. The same, with the cortex completely dissolved. *v c*, contractile vacuoles.
Fig. 13. The same, in slow flow in the direction indicated by the arrow. *r s*, the newly reconstituted cortex.
Fig. 14. Another example, in which the cortex has just become liquefied, but is still retained at one spot, together with two pseudopodia.
Fig. 15. An *Amœba* in which the cortex has dissolved before two pseudopodia (*ps*) were retracted. These become liquefied soon afterwards. In this and
Fig. 16 the granular protoplasm is sharply separated from the hyaline zone. This, however, only lasts for a few moments, to give place to the state in fig. 12.
Fig. 17. An *Amœba* in which the liquefaction of the cortex had just commenced on one side, treated with osmic acid. The cortex (*r s*) appears finely punctate, as also the hyaline sarcode; the nucleus at *n*.

XV.—*Contributions towards a General History of the Marine Polyzoa.* By the Rev. Thomas Hincks, B.A., F.R.S.

[Continued from vol. viii. p. 136.]

[Plate V.]

IX. FOREIGN CHEILOSTOMATA (Miscellaneous).

Family **Flustridæ**.

Flustra, Linnæus.

Flustra dentigera, n. sp. (Pl. V. figs. 7, 7 *a*.)

Zoarium of a rather dark-brown colour and a somewhat waxy appearance, with a narrow smooth edging, dividing dichotomously into tall, linear, strap-like segments, expanding very slightly upwards, which are not divergent, but continue in close proximity throughout their length. *Zoœcia* alternate, elongate, arched above and somewhat expanded, usually narrowing slightly below the middle, a line of nume-